ELSEVIER

Experimental Hematology

Experimental Hematology 2010;38:1–2

LETTER TO THE EDITOR

Mouse MAPC-mediated immunomodulation: Cell-line dependent variation

Adoptive therapy with mesenchymal stem cells (MSC) is being developed as a promising approach in the treatment of graft-vs-host disease (GVHD). In vitro, MSC exhibit strong immunomodulatory properties toward alloantigen-induced T-cell responses [1], and although evidence from in vivo mouse models is conflicting [2] in clinical practice, systemically delivered MSC have been proven effective in reducing GVHD [3]. Multipotent adult progenitor cells (MAPC), a recently described population of nonhematopoietic bone-marrow–derived stem cells with a broader differentiation capacity than MSC, have currently received attention as an alternative source for immunomodulatory cell therapy. Human MAPC can be expanded for >60 population doublings, which should allow use of a single-donor cell bank to treat large patient numbers. Kovacsovics-Bankowski et al. have reported that clinical-scale–expanded rat MAPC, following systemic delivery, can inhibit rat GVHD lethality [4], and the first clinical trial with large-scale–expanded human MAPC (MultiStem) for GVHD has been initiated. More recently, Highfill et al. reported that mouse MAPC (mMAPC) suppress in vivo T-cell alloresponses and GVHD provided they are delivered intrasplenically at the site of initial T-cell alloactivation [5].

We have studied the immunomodulatory behavior of *mMAPC-2*, an mMAPC clone we described previously, to express significant amounts of the embryonic transcription factor *Oct4* (5–10% of murine embryonic stem cells [mESC]), as well as primitive endoderm transcripts (*Gata4;Gata6;Sox7;Sox17*) [6,7], and to differentiate to cells with endothelium, hepatocyte, and neural progenitor characteristics [6,7]. This MAPC clone is similar to the clone reported on by Highfill et al. [5]; however, although our in vivo data underscore the potential of mMAPC for GVHD-immunomodulation, the in vitro data indicate that mMAPC also can exert immunostimulatory actions in an inflammatory milieu.

Offprint requests to: An D. Billiau, M.D., Ph.D., Laboratory of Experimental Transplantation, Campus Gasthuisberg O&N I Box 811, Herestraat 49, Leuven 3000, Belgium; E-mail: an.billiau@med.kuleuven.be

Materials and methods

To investigate the in vivo immunomodulatory effects of *mMAPC-2 (C57BL/6 origin)*, we used the *C57BL/6(H-2^b)→CB17/SCID(H-2^d)* major histocompatibility complex (MHC)–disparate model of local GVHD (popliteal lymph node assay) and systemic GVHD. In popliteal lymph node assay, we injected 10×10^6 purified C57BL/6 T-cells (pan-T-cell kit, MACS; Miltenyi Biotec Gmbh, Germany) with 5×10^6 *mMAPC-2* in the footpad of natural killer cell–depleted (anti-asialoGM1, Wako, Germany) CB17/SCID mice; in the contralateral footpad only C57BL/6 T cells were injected. After 1 week, TCR-V$\beta3^+$-expressing alloreactive T-cell frequency was determined in popliteal lymph nodes using flow cytometry (anti-mouse TCR-Vβ3-phycoerythrin and CD3-peridinin chlorophyll monoclonal antibody; Becton Dickinson Biosciences, Erembodegem, Belgium). For induction of systemic GVHD, natural killer cell–depleted CB17/SCID mice received intravenous injections of 0.5×10^6 purified C57BL/6 T-cells on day 0, with or without co-injected 0.5×10^6 *mMAPC-2* on days 0 to 21 (5 injections), 0 to 14 (5 injections), or 1 to 13 (4 injections) (3 independent experiments); GVHD outcome was monitored based on body weight and clinical GVHD score. In vitro, the immunomodulatory effect of *mMAPC-2 on C57BL/6(H-2^b)→BALB/c(H-2^d)* mixed lymphocyte reaction (MLR) was compared to that of mMSC (D Prockop, Tulane University, New Orleans, LA, USA) and mESC (both C57BL/6 origin). Mitomycin-C–treated mMAPC-2, mESC, mMSC were added on day 0 at four different suppressor-to-effector (S:E) ratios (1:100 to 1:1) to the MLR (MACS-purified (C57BL/6) CD4$^+$ T-cells ↔ (MACS-purified + mitomycin-C–treated) (BALB/c) splenic CD11c$^+$ dendritic cells). After a 4-day culture, T-cell-proliferation was measured (^{3}H-thymidine incorporation or carboxy fluorescein succinimidyl ester dilution in the viable cell population (7-amino-actinomycin D and Annexin-V).

Results and discussion

In vivo, *mMAPC-2* efficiently suppressed alloreactive T-cell expansion in popliteal lymph node assay: mean TCR-V$\beta3^+$-expressing alloreactive T-cell frequencies were 19.14% ± 2.8% standard error (SE) and 6.79% ± 2.6% SE in the T cell, respectively. T-cell + mMAPC-2–injected limbs ($p = 0.001$, Wilcoxon rank-sum test; n = 8/group, n = 6 independent experiments). However, in the systemic GVHD model, both the T-cell + mMAPC-2–treated and the T-cell–only group

0301-472X/10 $–see front matter.
doi: 10.1016/j.exphem.2009.10.009

developed a similar degree of severe GVHD. The failure of intravenously delivered *mMAPC-2* to suppress systemic GVHD in our model and that of Highfill et al. [5] is consistent with previous mouse studies on the failure of MSC to inhibit systemic GVHD [2]; however, our data confirm the potential of mMAPC to modulate in vivo graft-vs-host reactivity, provided they are delivered locally at the site of initial T-cell alloactivation. In contrast to the in vitro data obtained by Highfill et al. [5], however, we documented that *mMAPC-2* consistently exert a bimodal modulatory effect on in vitro alloreactive T-cell proliferation, with an immunostimulatory effect at low S:E ratios (e.g., 5% and 66% stimulation at S:E 1:100, 1:10, respectively) and a suppressive effect at high S:E ratios (e.g., 81% suppression at S:E 1:1) (one of five independent assays). In contrast, simultaneously tested mMSC and mESC exerted a dose-dependent suppressive effect, consistent with literature (e.g,. 38%, 38%, 81% for mMSC and 0%, 32%, 83% for mESC at S:E 1:100, 1:10, and 1:1) [1,8]. An identical bimodal immunomodulatory pattern was documented with *mMAPC-1*, a clone with transcriptome and functional properties identical to *mMAPC-2* [6]. When *mMAPC-2* were separated from the MLR by a 0.4-μm pore size membrane (Transwell; Corning Incorporated, Corning, NY, USA), the immunostimulatory effect disappeared while immunosuppressive effects were preserved, indicating that mMAPC-mediated immunostimulation is contact-dependent and suppression is contact-independent. In contrast to what is known for mouse and human MSC [1], and in contrast to Highfill et al. [5], inhibiting PGE2 and inducible nitric oxide synthase synthesis did not inhibit *mMAPC-2* immunosuppression. The mechanisms involved in the immunostimulatory effects are as yet undetermined.

In summary, we conclude that Oct4^{high} *mMAPC-2* suppress in vivo graft-vs-host reactivity provided they are delivered locally at the site of initial T-cell alloactivation, confirming the potential of mMAPC for GVHD immunosuppression as recently reported by Highfill et al. [5] These findings emphasize the critical role of stem-cell homing to sites of T-cell alloreactivity and this has important implications for future clinical applications. However, as evident from the contrast between our findings and those of Highfill et al. [5], similar MAPC-populations exhibit functional differences in vitro, and our data particularly suggest that immunostimulatory effects may hold safety concerns for in vivo application. There is also evidence that murineMSC are not intrinsically immunoprivileged: a bimodal in vitro immunomodulatory effect has also been reported for human and mouse MSC [9] and, moreover, there is evidence in mice that MSC, under specific conditions, can induce a memory T-cell response in allogeneic hosts [10]. In view of the difficulty to correlate in vitro and in vivo immunomodulatory effects of e.g., MSC, our data warrant that functional differences between similar stem cell types should be considered when contemplating the use of a given cell-line for immunomodulation in specific disease-contexts.

Acknowledgments

A.L. is a doctoral fellow of the Institute for the Promotion of Innovation by Science and Technology in Flanders. L.D.S. is a doctoral fellow and A.D.B a postdoctoral fellow of the Flanders National Fund for Scientific Research. We thankfully acknowledge Martine Geraerts for help on MAPC cultures and Caroline Lenaerts for technical support.

Conflict of Interest Disclosure

No financial interest/relationships with financial interest relating to the topic of this article have been declared.

Ariane Luyckx[a,*]
Lien De Somer[a,*]
Omer Rutgeerts[a]
Mark Waer[a]
Catherine M. Verfaillie[b]
Stefaan Van Gool[c]
An D. Billiau[a]
[a]*Laboratory of Experimental Transplantation*
[b]*Stem Cell Institute*
[c]*Laboratory of Experimental Immunology, University of Leuven, Leuven, Belgium*

*Drs. Luyckx and De Somer contributed equally to this work.

References

1. Nauta AJ, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. Blood. 2007;110:3499–3506.
2. Sudres M, Norol F, Trenado A, et al. Bone marrow mesenchymal stem cells suppress lymphocyte proliferation in vitro but fail to prevent graft-versus-host disease in mice. J Immunol. 2006;176:7761–7767.
3. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. Lancet. 2008;371:1579–1586.
4. Kovacsovics-Bankowski M, Streeter PR, Mauch KA, et al. Clinical scale expanded adult pluripotent stem cells prevent graft-versus-host disease. Cell Immunol. 2009;255:55–60.
5. Highfill SL, Kelly RM, O'Shaughnessy MJ, et al. Multipotent adult progenitor cells (MAPC) can suppress graft-versus-host disease via prostaglandin E2 synthesis and only if localized to sites of allopriming. Blood. 2009;114:693–701.
6. Ulloa-Montoya F, Kidder BL, Pauwelyn KA, et al. Comparative transcriptome analysis of embryonic and adult stem cells with extended and limited differentiation capacity. Genome Biol. 2007;8:R163.
7. Serafini M, Dylla SJ, Oki M, et al. Hematopoietic reconstitution by multipotent adult progenitor cells: precursors to long-term hematopoietic stem cells. J Exp Med. 2007;204:129–139.
8. Koch CA, Geraldes P, Platt JL. Immunosuppression by embryonic stem cells. Stem Cells. 2008;26:89–98.
9. Le Blanc K, Rasmusson I, Götherström C, et al. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. Scand J Immunol. 2003;57:11–20.
10. Nauta AJ, Westerhuis G, Kruisselbrink AB, et al. Donor-derived mesenchymal stem cells are immunogenic in an allogeneic host and stimulate donor graft rejection in a nonmyeloablative setting. Blood. 2006;108:2114–2120.